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Abstract:	Mediterranean farmers have a really limited choice for winter crops to put in rotation with cereals, thus creating big challenges for weed and disease management. Crop diversification has undisputable environmental benefits and plays a central role in the agroecological transition toward sustainable and resilient farming systems. Among other crop candidates, safflower (Carthamus tinctorius L.) is recently attracting the attention of Mediterranean farmers, due its broad environmental suitability, low input needs, high plant vigor, also in marginal soil conditions, and tolerance to low temperatures. Thus, in the whole Mediterranean basin, safflower could be grown with a winter cycle, differently than sunflower (Helianthus annuus L.). The availability in the market of high-oleic safflower varieties tremendously enlarges the applications of its oils, easily meeting the needs of the domestic bio-based industry. Aiming at evaluating the feasibility of high-oleic safflower as a winter oilseed crop in the Mediterranean region, a multi-year and multi-location study has been carried out, across multiple growing seasons (2019-2021), at eight locations across Emilia-Romagna (ER) and Tuscany (TU) regions (Italy), traditionally devoted to winter cereal cultivation. In each region, the locations were chosen as representative of optimal, mean, and marginal conditions. The trials were managed as on-farm experiments by local farmers, to define safflower suitability to available equipment and practices. All trials were rainfed and carried out under low input agronomic management and using mechanical weed control. The safflower seed yield was not affected by growing region (grand mean: 1775 kg DM ha -1), while 1000-seed weight and seed oil content were significantly influenced by growing environment. In particular, safflower produced significantly heavier seeds in Emilia Romagna (40.8 vs. 38.2 g, ER vs. TU, respectively, $P \leq 0.05$), while seed oil content was higher in Tuscany (TU vs. ER, 40.3 vs. 36.1% DM respectively, $P \leq 0.05$). Safflower confirmed its compositional stability with oleic acid representing >75% of total fatty acids, but, again, some differences were revealed between regions, with ER having significantly higher oleic acid content than TU (78.8 vs. 75.9%, ER vs. TU, respectively). High oleic safflower, grown in winter, confirmed to be an interesting opportunity for Mediterranean farmers who are willing to differentiate

	their rotations while producing an oilseed crop with several biobased applications and able to increase local production of vegetable oil and protein.

Dear Editor

All the comments of the Editor and of the Reviewer #1 have been taken into account and are answered in this document. Answers to Reviewers' comments are reported in red.

Editor's comments:
In addition to the comments of the reviewers, follow the checklist below and modify your manuscript accordingly. If an item on the checklist does not apply to your manuscript, just skip it. Write in red all changes made to your manuscript in next revision, do not use Word Track changes

1) Add continuous line numbers to your document OK
2) Tables and figures go after references. Do not embed them in the text. OK
3) Title: Avoid low impact words such as 'effects of', 'influence of', 'characterization of', etc., any part of the title. Title must be declarative, descriptive or a question. Google how to write a high impact title for a scientific publication OK
4) Do not use abbreviations in highlights OK
5) Add one sentence of rationale to the beginning of your abstract OK
6) All acronyms must be spelled out in the abstract OK
7) Write in third person, avoid personal pronouns, such as we, they, you, I, or our , their, yours OK
8) Abstract must have rationale, objective, materials and methods and conclusions. First sentence must be a rationale. Do not write the words: rationale, methods, results in the abstract OK
9) Add in your manuscript your reply to comments where the question was raised. A future reader of your publication might have a similar question. OK
10) Common names of plants, animals, fungi, etc. must be followed by the Latin name the first time the common name is used. Latin name must include Authority example: maize (Zea mays L.) OK
11) Do not start sentences with abbreviations or numbers Abbreviation for number is no. OK
12) No space between the unit and Celsius symbol, correct all OK
13) replace 'compared to' with 'compared with', correct all OK
14) Equations must have the form y=a + bx , correct text , figures and tables. OK
15) All statistical parameters y, x, n, r2, P, p...etc must be in Italics in text figures and tables. Use small case r2 for linear equations , R2 is used only for non-linear regressions OK
16) Use significant digits only in values and use. period for decimal separation check all tables and Figures OK
17) Replace ppm for mg/kg or mg/L OK
18) For currency use only US dollars and Euros OK
19) Tables, make sure the independent variables are in the first column. You might need to transpose columns and rows, dependent variables in columns 2 to n with the unit below. OK
20) No bold text or values in tables OK

21) Justify first column of tables to the left OK
22) Tables: Units go below header lines. Delete units from captions. Correct all tables OK
23) Format your tables to journal style. No vertical lines and only 3 horizontal lines, top, bottom and line below header. OK
24) Only one table per page after references. OK
25) Move Figures to the end of the text after tables , one figure per page with the caption below the Figure OK
26) Tables must stand alone, indicate the meaning of all abbreviations used on the table in a footnote. Footnotes indicators must have small case letter in italics and superscript (a,b,c or x, y z) do not use * for footnotes. One line per footnote below the table. OK
27) Check references format (Johnson, 1993), Johnson and Smith, 1993), (Johnson et al., 2003). For references list use ICP reference formatting. Journal titles must be abbreviated using the standard abbreviation, which you can find OK
on https://www.library.caltech.edu/journal-title-abbreviations. Example: Ind. Crops Prod. Also, add doi for the reference if available. OK
a. Delete 'and' before last author. Delete 'parentheses' from year. OK
b. Write article title with all words in small case letters do not capitalize words that do not need to. OK
c. Latin names in titles must be in Italics OK
28) For dates format use: 12 August 2016, not August 12th, 2016 OK
29) All units and values are separated by space except % and Celsius degree symbol oC examples: 15 mL, 20 min, 600 nm, 1000 kg/ha, 46%, 20oC, 8.60 g OK
30) Use a comma before the final item in a list of three or more items. For example: "Cores were inside plastic liners, capped, and stored on ice…" OK
31) For ordinal numbers use the word first, second, third, fourth not 2nd,3rd,4th OK
32) All elements are standard abbreviations; do not need to introduce N, P, K, Mg, Cd, Pb, Zn, etc. OK
33) Common standard abbreviations hour= h, minutes= min, seconds= s, liters= L, grams= g kilogram = kg, for metric tons use Mega grams Mg/ha, for temperature use ℃. OK
34) For numbers 1-10 write them out (one, two,… ten) unless they follow an unit. Example: three replicates OK

Rev #1

The experiment was conducted at different locations in two regions of Italy differing in temperature and other growing parameters to test the performance of an oleic variety of safflower and the effects of regions, locations and weather, and agronomic aspects on fatty acids and oil content. The study has shoed that the oleic variety has stably expressed high oleic acid (75.9-78.8%), high oil content (>35%). The study would help in enhancing the area under oleic safflower. The methodology of statistical analysis, oil content, and fatty acids estimation was correct but the methodology of field experimentation was confusing. It should have been presented in a straight way like 'the experiment was conducted in Italy in two different regions at so… and so… locations in so… and so… years. The emphasis should be on the difference

between different regions and different locations within a region regarding the performance of the oleic variety so that one could identify which region/locations are more suitable or are the selected regions suitable for growing oleic variety. The results and discussions should have been done accordingly. It is well known that year x location/region will affect the crop growth depending on the growing conditions such as temperature, rainfall, etc, especially under rainfed conditions.

A response to this comment is given below

The importance put on growing seasons is confusing. How many seasons are there in Italy for growing winter crops?

Sorry authors cannot address this comment "How many seasons are there in Italy for growing winter crops?" since we didn't understand which was the request.

The experiment at the same location was planted at different dates in different years; the difference in planting date at the same location between years had ranged from 4 (CA) to > 30 days (SL, SPG) (Table 2). The reasons for taking up the experiment at different planting dates in different years should be explained.

The reviewer is right but being the trials established at farmers' farm it was not possible to maintain the sowing date too similar across growing season since each farmer decided autonomsly which was the best condition for sowing with respect of his soil and available equipment as well as of the weather conditions. This concept has been reported also in the text L76.

Introduction

Do the eight locations selected for testing the performance of oleic safflower in Italy represent the entire Mediterranean region? Explicitly give the countries for which this study is aimed to grow oleic safflower since the Mediterranean region is a big one.

The reviewer is right a better circumstantiation of the Mediterranean region and related climate in which safflower was tested is now included in the introduction section. (L 68)

Page 2 and 3

Line 44-45: Change the sentence " due to selection of weeds to common herbicides -------" to " to protect crops from herbicide-resistant weeds and high build-up soil-borne diseases' inoculum in the soil."

The sentence has been changed as suggested. L 47-48

Line 46: insert is in between diversification and one of the cornerstones; combined corner stones into one word 'cornerstones'.

The sentence has been modified as the rewiever suggested L50

Line 48: Change 'restrained to 'limited'.

The word was changed L52

Line 49: change ‘these’ to ‘the’.

The word “these” was replaced with “the” L53

Line 50 and 66: remove ‘:’ after ‘as’.

The “:” was removed L54, L70

Materials and methods

Sub-title: Change capital “C’ into small “c’ in characteristics.

The capital C was changed into small as suggested L79

Line73: What is ‘MAS Seeds’, explain

MAS seeds is the Italian seed company that provided the safflower seeds for the trials. L80

Page 4

Line 81; Change (OZ-1-2-3) into (OZ-1, OZ-2, and OZ-3).

done. L85

Line 97: give reference for Bray method.

The reference for Bray method was added to the text. L102

Line 99: what is ‘by 1.724’, explain it.

1.724 is a costant factor, commonly used for the estimation of organic matter in soils. As the reviewer suggested, the explanation of this costant factor has been added to the text. L104

Line 101: change ‘established’ to ‘sown’.

done. L107

Line 102-103: Mention the standard low-input practices, applied by local farmers, and the changes that deviated from farmers’ practices in your study for clear understanding.

As suggested the definition of low input practices has been included in the manuscript in L 109-110

the study was carried out in two contiguous years (2019, 2020) at Tuscany, and 2020 and 2021 in Emilia Romagna, and one year (2020) in LA and OZ1; does Emilia Romagna covers the OZ2 and OZ3.

Line 104-106: The sentence is confusing. The experiment was conducted for two years and one year at different locations, not three years/growing seasons as mentioned in the Abstract and Introduction. Correct this. In the Abstract, it was mentioned that a multi-year and multi-location study has been carried out, across three growing seasons (2019-2021), it is misleading that the experiment was conducted at the eight locations in three growing seasons or years.

The reviewer is right, so the content of the abstract has been revised L 21 and also the M&M section L81 and Table 1

You may change the sentence to " the experiment was conducted for two years (2019-2020 or 2020-2021) at so and so …… locations in so and so……region and one year (2020) at so and so …… locations in so and so ….. region".

The reviewer is right but what he/she is asking is already correctly reported in L 81 and Table 1

Page 5

Line 110: How Tbase 5º C was considered just considering reference Mirshekari et al., 2013 or was calculated for your locations?

Authors assumed a Tbase of 5°C for the calculation of GDD as retrieved from the literature and not determined by each experiment, since this was not the focus of the study.

Give more emphasis on the utilization of GDD to determine planting dates and yields compared to the calendar year in your experiment to facilitate an efficient fertilizer and insecticide application schedule in each region.

Being safflower an almost new crop for Italy authors decided to use GDD as an easy way to compare the growth of the tested genotype across the experimental sites, but GDD were not used to define optimal dates for the management of the crop in this study, but for sure as suggested by the revierwer they might be used so in the future, if the crop will spread.

Change 'number of heads' to 'number of capitula' across the manuscript as the fruiting part in safflower is called capsule (singular) or capitula (plural).

Authors changed as suggested heads into capitula throughout the manuscript, but the singular of capitula is capitulum while capsule is a completely different structure and does not apply to safflower.

Line 121: Convert thousand seed weight into 1000-seed weight' cross the manuscript.

As suggested, TKW was replaced throughout the text

Define ISTA standards (2005) for assessing 1000-seed weight in safflower

The reference to support the method is included in the text, and in L129-131, authors included a brief description of the method as requested.

Give measuring units (such as cm, g, kg/ha, %) within bracts after plant height, 1000-seed weight, seed yield, oil yield, and oil content.

Done L125-126

2.3 Seed quality analysis

2.3.1. Seed oil content

Line 127: Change the unit mL to ml everywhere.

Authors preferred not to keep this suggestion since the Editor suggested the opposite in accordance to ICP formatting rules

Line 128: add ‘an’ before ‘organic solvent’.

Done L138

Page 6

2.3.1 Oil analysis

Give the reference for the Soxhlet extraction method

The Soxhlet is the apparatus used for the extraction and the method is already reported so authors think it is not necessary to add a reference for the method.

Line 135: Is it Seed yield or oil yield? Oil yield is derived by [(seed yield × oil content) / 100]. Correct it.

Done the sentence has beend rephrased to make it clearer L145-146

Page 7

Statistical analysis

Line 165: What is TKW, expand it.

TKW was replaced with 1000-seed weight as suggested

Line 166: Expand SFA, MUFA, PUFA, indicate linoleic and oleic acids come under which category of fatty acids, MUFA or PUFA, and also give what are other saturated, mono, and polyunsaturated fatty acids were assessed.

As suggested, abbreviation paragraph has been included in the manuscript just before the abstract. Then in L177-179 all the FAs included in each group are now reported

Line 167: change LSD’s into LSD

Done throughout the manuscript

- Line 168: Change Principal Components to Principal Component.

Done.

- Line 171-172: Principal component analysis (PCA) was carried out on the correlation matrix (covariance matrix of the standardized variables). What does the ‘covariance matrix of the standardized variables’ mean? Using the correlation matrix is equivalent to standardizing each of the variables (to mean 0 and standard deviation 1). Have you converted the covariance matrix into a correlation matrix? Clearly explain which matrix was used and why?

Authors highly appreciated this comment, and we want to clarify as much as possible this misunderstanding. First, the covariance matrix was used and then the correlation coefficient was calculated, dividing the covariance of the variables by the product of the standard deviations of the same values. So yes, the covariance matrix has been converted into a correlation matrix and the PCA was then performed on the standardized data. To avoid confusion, the sentence in brackets has been deleted. L184-197

- Have you used the mean of all locations and years for each variable used in PCA? Explain the methodology properly.

Reviewer is right, the means of all locations and years for each variable have been used in PCA. Each variable was represented by single fatty acid. As the reviewer suggested, this information has been added to the text. L184-197

- Line 180-181: Give references indicating the good Hopkins score (<0.5) or >0.5%) since there are contradictory opinions on Hopkins scores, some say that > 0.5 is a "clusterable" data set, while anything <0.5 is not. Others say that anything above or below 0.5 is "clusterable" data.

As the reviewer suggested, references about Hopkins statistic has been added to the text. L194-195

Page 8

Line 188: CW99 OL is not a hybrid, it is an American high oleic safflower variety (Collins, H, 2013. Safflower Production in Eastern Washington- Background History……).

Thank you for this interesting comment, "hybrid" has been delayed from the text L202

3. Results

3.1 Meteorological conditions and crop cycle length

Replace 'd' with 'days' ex: 190 d into 190 days (Line no.189).

Done, also throughout the entire manuscript

Though the study clearly showed that the regions selected were suitable for oleic variety cultivation the results and discussion parts may be rewritten and resubmitted as suggested in the first paragraph of the reviewer's comments.

We agree with the reviewer but one of the main goals of the study was to demonstrate the operational feasibility of safflower at farm scale in northern and central Italy, rather than identifying the most suitable growing environment. In fact, in each region different types of soils (mean, marginal and highly productive) were compared, in order to produce a very reliable dataset on the feasibility of winter safflower in Italy. Notwithstanding, we improved the conclusions in order to better respond to this comment of the reviewer (L 395-399).

Highlights

- High-oleic safflower was grown at farm-level in north and central Italy for two consecutive growing seasons
- Oil yield exceeded in the best condition 900 kg ha^{-1} of oil.
- Safflower confirmed its compositional stability with oleic acid representing >75% of total fatty acids
- High-oleic safflower appeared a promising alternative to winter cereals for Mediterranean farmers

Safflower (*Carthamus tinctorius* L.) a winter multipurpose oilseed crop for the Mediterranean region: lesson learnt from on-farm trials

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Abstract

Mediterranean farmers have a really limited choice for winter crops to put in rotation with cereals, thus creating big challenges for weed and disease management. Crop diversification has undisputable environmental benefits and plays a central role in the agroecological transition toward sustainable and resilient farming systems. Among other crop candidates, safflower (*Carthamus tinctorius* L.) is recently attracting the attention of Mediterranean farmers, due its broad environmental suitability, low input needs, high plant vigor, also in marginal soil conditions, and tolerance to low temperatures. Thus, in the whole Mediterranean basin, safflower could be grown with a winter cycle, differently than sunflower (*Helianthus annuus* L.). The availability in the market of high-oleic safflower varieties tremendously enlarges the applications of its oils, easily meeting the needs of the domestic bio-based industry. Aiming at evaluating the feasibility of high-oleic safflower as a winter oilseed crop in the Mediterranean region, a multi-year and multi-location study has been carried out, across multiple growing seasons (2019-2021), at eight locations across Emilia-Romagna (ER) and Tuscany (TU) regions (Italy), traditionally devoted to winter cereal cultivation. In each region, the locations were chosen as representative of optimal, mean, and marginal conditions. The trials were managed as on-farm experiments by local farmers, to define safflower suitability to available equipment and practices. All trials were rainfed and carried out under low input agronomic management and using mechanical weed control. The safflower seed yield was not affected by

growing region (grand mean: 1775 kg DM ha^{-1}), while 1000-seed weight and seed oil content were significantly influenced by growing environment. In particular, safflower produced significantly heavier seeds in Emilia Romagna (40.8 vs. 38.2 g, ER vs. TU, respectively, $P \leq 0.05$), while seed oil content was higher in Tuscany (TU vs. ER, 40.3 vs. 36.1% DM respectively, $P \leq 0.05$). Safflower confirmed its compositional stability with oleic acid representing >75% of total fatty acids, but, again, some differences were revealed between regions, with ER having significantly higher oleic acid content than TU (78.8 vs. 75.9%, ER vs. TU, respectively). High oleic safflower, grown in winter, confirmed to be an interesting opportunity for Mediterranean farmers who are willing to differentiate their rotations while producing an oilseed crop with several biobased applications and able to increase local production of vegetable oil and protein.

Keywords: seed yield; oleic acid; seed oil content; low-input management, on-farm experiment; crop diversification.

Abbreviations: FA, fatty acid; MUFA, Monounsaturated fatty acid; PUFA polyunsaturated fatty acid; SFA, saturated fatty acid; C18:1, oleic acid; C18:2, linoleic acid; TSW, 1000-seed weight; GDD, growing degree days, ER, Emilia Romagna; TU, Tuscany.

1. Introduction

In the Mediterranean region only winter cereals, i.e. wheat (*Triticum* spp.) and barley (*Hordeum vulgare*), are extensively grown with an autumn cycle, without any feasible alternatives at large scale, mainly in relation to specific environmental conditions and well-established agronomic practices. So, differently from the rest of Europe, in the Mediterranean region winter oilseed rape (*Brassica napus* L. var. Oleifera) is only seldom grown due to its susceptibility to drought, poor adaptability to soils with low fertility, and the lack of specific breeding programs for this area. Thus, Mediterranean farmers mostly rely on cereals as winter crops, this making weed management highly challenging to protect crops from herbicide-resistant weeds (i.e. *Lolium* spp.) and high build-up soil-borne diseases inoculum in the soil (i.e., *Fusarium* spp.). Furthermore, the limited number of winter crop options makes the situation for organic farmers even more complicated, being crop diversification is one of the cornerstones of organic practices to reduce weed and disease pressure and promote yield. To meet the needs of Mediterranean farmers some new winter crops are trying to enter their typical

cropping systems but the agronomic knowledge of these new species is still very limited. In relation to the domestic shortage of vegetable oil and protein, some of the new winter crops suitable for the Mediterranean region are oilseeds, such as camelina (*Camelina sativa* L. Crantz), carinata (*Brassica carinata* L.), and more recently safflower (*Carthamus tinctorius* L.). Safflower is a native species of Near East Asia, and it was firstly reported in Europe 5800 BC (Marinova and Riel, 2009). It belongs to the *Asteraceae* family, like sunflower (*Helianthus annuus* L.), the most widespread oilseed crop in the Mediterranean basin. Differently from the latter, safflower is tolerant to low temperature and could grow with an autumn/winter cycle in such environment. This trait, together with the other traits of interest such as the resistance against bird predation, the negligible seed losses due to shattering (Mayerhofer et al., 2011) and the early soil cover of winter-sown crop with reduced risk of N-leaching and soil erosion, confers safflower an outstanding potential to become a potential winter oilseed crop for the Mediterranean climate. The feasibility to grow safflower with a winter cycle prevent, or at least dramatically reduce, the possible occurrence of drought stress at flowering stage, which is the only one very sensitive in this species (Koutroubas and Papakosta, 2010; La Bella et al., 2019). Furthermore, safflower is a multi-purpose crop being able to source natural red (carthamin) and yellow dyes from its petals (Patanè et al., 2020), but also oil (≈35-40%) and protein (≈20%) from its seeds (Zanetti et al., 2013). Recently breeding effort has led to the selection of high oleic safflower hybrids (Golkar & Karimi, 2019), which are more suitable to biobased applications (Nogales-Delgado et al., 2021), thus further promoting the potential of this crop as a non-food alternative to sunflower, possibly expanding further the growth basin of oilseed crops in Mediterranean Europe, particularly under North Mediterranean climatic conditions (Metzger et al., 2005). High oleic oils have increased oxidation stability (Merrill et al., 2008), compared with other vegetable oils, and they adapt well to several well-established chemical processes, able to source various biobased products, such as biolubricants, bioherbicides, bioplastics, etc. (Nogales-Delgado et al., 2021; Zhu et al., 2016).

The future scale up and diffusion of a new crop in a new environment needs to encompass the design of sustainable cropping systems, combining empirical and scientific knowledge (Leclere et al., 2018; Toffolini et al., 2016). At this scope a multi-year and multi-location trial has been established across eight different sites across Italy, in Emilia-Romagna and Tuscany regions, with the aim to assess the productive potential of the crop, and to demonstrate its feasibility at farm level, since all the trials were run under real operation conditions by local farmers.

2. Materials and Methods

2.1. Site characteristics and agronomic management

The commercial safflower high oleic variety, CW99OL (provided by MAS Seeds Italia, Italy), was tested in eight farmers' field trials during multiple growing seasons (see Table 1 for details on the growing year/site), in different pedo-climatic conditions of central and northern Italy. Fields were located in hilly and plain areas of Emilia Romagna and Tuscany regions, within an area ranging from 43.27-44.32" N latitude, and 10.18-11.28" E longitude. The cultivation sites of Tuscany were located at Santa Luce (SL), Fauglia (FA), San Piero a Grado (SPG), and Larciano (LA). While in Emilia Romagna one trial was located at Cadriano (CA) and three at Ozzano dell'Emilia (OZ-1, OZ-2, and OZ-3) (Table 1). Santa Luce (SL) site was located in the hilly area of Pisa province (Tuscany) with 15% slope and it was characterized by alkaline, calcareous, clay-loamy soil with a low content of available phosphorus and a good level of exchangeable potassium. Fauglia (FA - located at the beginning of the hilly area of Pisa, with 20% slope) and Larciano (LA - near the Fucecchio Marshes, Pistoia province, 0% slope) sites were characterized by a sandy-loamy soil with sub-acid pH and a very low level of available phosphorus. San Piero a Grado (SPG) field was located in the Pisa coastal plain, with alluvial deep loam soil and alkaline reaction and low level of SOM and total nitrogen. SL and FA sites can be considered as marginal land, as defined by Elbersen et al. (2017). Emilia-Romagna locations were all in the Bologna province, but representing different pedological conditions, which are quite typical of the whole region. Cadriano was characterized by sub-alkaline, loamy soil with good content in exchangeable potassium and total nitrogen. Ozzano dell'Emilia sites were characterized by three different slope levels: OZ-1 situated in a plane field with a clay-loam soil and sub-alkaline pH, good SOM content and total nitrogen levels, OZ-2 and OZ-3 were sloppy sites with 15% and 25% slope respectively, those sites can be considered as marginal land, as defined by Elbersen et al. (2017). Soil physical and chemical characteristics were assessed at the beginning of the experiment, collecting the soil samples at 30 cm depth in each field. Site description and physico-chemical soil characteristics are presented in Table 2. Soil pH determination was performed on a 1:2.5 soil: water suspension following McLean procedure (1982). Total nitrogen was evaluated using the macro-Kjeldahl digestion procedure (Bremner and Mulvaney, 1982), available phosphorus by colorimetric analysis using the Olsen (Olsen and Sommers, 1982) or Bray method (Bray and Kurtz, 1945) according to soil pH value. Soil organic matter was estimated by multiplying the soil organic carbon concentration, measured using the modified Walkley–Black wet combustion method (Nelson and Sommers, 1982), by a constant factor. The factor used is 1.724 assuming that soil organic matter is made up of 58% C (Tabatabai, 1996). Main information regarding the adopted agronomic management (i.e. soil tillage, sowing method, fertilization, sowing date and harvest time) are presented in Table 1. All the trials were managed by local farmers under real operational conditions in order to get as much as possible reliable data on safflower suitability to northern and central Italy. Safflower was sown

in large strips of 500-1000 m^2 at each farm, and the agronomic management was implemented differently at each experimental site according to standard low-input practices (i.e., by adopting organic fertilization or very low amount of mineral fertilizers, and mechanical weeding instead of chemical control), applied by local farmers, and specific soil needs. Sowing took place in winter between the beginning of January and the end of March, while harvest was carried out between the end of July and the beginning of August. The studied growing seasons were 2019 (TU_GS1) and 2020 (TU_GS2) in Tuscany, and 2020 (ER_GS1) and 2021 (ER_GS2) in Emilia Romagna; in LA and OZ-1 the experiment was carried out only in one season (i.e., 2020). For each study site and growing season, main daily meteorological data (i.e. minimum and maximum temperature, and precipitation) were recorded by weather stations located nearby the experimental sites (Table 3). Cycle length was calculated as the number of days from sowing to harvest. The accumulated growing degree days (GDD) were calculated, for each growing season, as follows:

$$GDD = \sum[(T_{max}+T_{min})/2 - T_{base}]$$

Where T_{max} and T_{min} are the maximum and minimum air temperature, respectively, and T_{base} for safflower was defined as 5°C (Mirshekari et al., 2013)

2.2 Surveyed parameters at harvest

At each study site, four representative areas of 4.5 m^2 in Tuscany, and 4 m^2 in Emilia Romagna were randomly sampled when safflower reached the maturity stage (stages 89-91 on the BBCH scale; Flemmer et al., 2015). Within each sampling area plant density (plants m^{-2}), seed and straw yield (kg DM ha^{-1}) were surveyed. Plant morphological traits and yield components, i.e., plant height (m), number of capitula per plant, number of lateral branches per plant were measured on a subsample of 15 plants from each sampling area. Residual moisture on seed and straw was evaluated by weighing representative subsamples before and after oven-drying at 105°C until constant weight was reached. Representative seed samples were preserved, and 1000-seed weight (g) was assessed according to ISTA (2005). The weight of eight replicates of 100 seeds each has been recorded. The mean weight of 100 seeds has been then used to calculate the weight of 1000 seeds.

2.3 Seed quality analysis

2.3.1. Seed oil content

About 30 g of safflower seeds were finely ground in a coffee grinder for 40 sec. An aliquot of 1.5 g of ground material was exactly weighed in a cellulose extraction thimble (22 × 80 mm) from Axiva Sichem Biotech (Delhi, India). The thimble was successively inserted in a 30 mL glass extractor and oil extraction was carried out in an in-line Soxhlet extraction unit (mod. R 306) from Behr Labor-Technik (Düsseldorf, Germany), using 60 mL of *n*-hexane as an organic solvent. Extraction was performed for two hours from the start of solvent siphoning into the round bottom flask placed on the heating element. Small pumice stones were added to the flask to avoid bumping of liquid following the increase of temperature. The extract containing the oily fraction was then filtered over anhydrous sodium sulphate in a 100 mL flat bottom flask and removed under reduced pressure at 30°C in a rotary evaporator. The residual oil was dried under a gentle nitrogen flow for 5 min keeping the flask in a water bath (50-55°C), exactly weighed, transferred by means of 5 mL of *n*-hexane/*i*-propanol 4/1 (v/v) in a 10 mL Teflon screw-cap glass tube, and stored at -18°C until fatty acid determination. Solvents used were of analytical grade. Oil yield (kg DM ha^{-1}) has been obtained by multiplying seed yield by seed oil content of each individual replicates.

2.3.2. Fatty acid analysis

Bound fatty acids were derivatised to the corresponding methyl esters (FAME) and then analysed by GC after a cold transmethylation performed on recovered safflower oil according to Christopherson and Glass (1969), with some modifications. About 20 mg of oil dissolved in *n*-hexane/*i*-propanol 4/1 (v/v) were dried under nitrogen flow in a glass conical tube placed in thermal heater (heater temperature: 40°C), exactly weighed, re-dissolved in 2 mL of *n*-hexane, stirred for 10 sec on a vortex stirrer and added with 0.05 mL of 2 M KOH in methanol. The mixture was then further kept on a vortex stirrer for 1 min and finally maintained at 4°C for 30 min to allow the separation of the upper organic layer from the lower methanolic phase. A volume of 0.33 mL of the supernatant fraction was diluted in 0.67 mL of *n*-hexane in a PP screw cap amber glass vials equipped with a silicone/PTFE septum and analysed by GC. A chromatographic system from Agilent Technologies (Santa Clara, CA, USA), made up of a gas chromatograph (mod. 7820A) equipped with an automatic liquid sampler (mod. G4567A) and a flame ionisation detector (FID) was used. A glass split liner packed with glass wool (i.d.: 4 mm) was installed in the injection port. Compound separation was carried out on a capillary column BPX70 (30 m × 0.25 mm i.d.; film thickness: 0.25 µm; stationary phase: 70% cyanopropyl polysilphenylene-siloxane) from SGE Analytical Science (Ringwood, Australia). Operating conditions were as follows: injection volume: 1 µL; injection mode: split; split ratio: 1/40; carrier gas (He) flow and linear velocity: 1.0 mL min^{-1} and 29.034 cm sec^{-1}, respectively; injector temperature:

240°C; oven temperature: 140°C for 2 min, from 140 to 220°C at 4°C min^{-1}, 220°C for 10 min; post-run temperature and flow: 240°C for 5 min and 1.5 mL min^{-1}, respectively; FID temperature: 250°C; hydrogen, air, and make-up flow: 30, 400, and 25 mL min^{-1}, respectively. A blank was performed injecting *n*-hexane every ten injections whereas every twenty injections oven temperature was raised from 140 to 240°C at 10°C min^{-1} and maintained at 240°C for 30 min under a constant gas carrier flow of 1.5 mL min^{-1} for column cleaning. GC traces were filed and processed by MassHunter Workstation Software (ver. B.06.00) from Agilent Technologies. Fatty acids were identified by matching peak retention times with those of a FAME standard mixture (GLC-463) from Nu-Check (Elysian, MN, USA). The relative amount of each fatty acid was determined from the ratio of its peak area to the sum of the peak areas of all fatty acids identified in the GC trace. Solvents used were of analytical grade.

2.4 Statistical analysis

Prior to ANOVA, the homoscedasticity of variance was verified with Bartlett's Test for $P \leq 0.05$. A two-way ANOVA was adopted to test the effect of the growing regions (Tuscany and Emilia Romagna) and the two growing seasons (GS1 vs. GS2) on the surveyed parameters and qualitative traits (i.e., plant height, plant density, straw and seed yield, 1000-seed weight, seed oil content, oil yield, oleic and linoleic acids, SFA, MUFA, PUFA). For FA group analysis, SFA included: stearic (C18:0), arachidic (C20:0), lignoceric acid (C24:0), behenic acid (C22:0), palmitic acid (C16:0); for MUFA: oleic (C18:1), eicosenoic acid (C20:1); for PUFA: linoleic acid (C18:2). When ANOVA revealed statistically different means, the LSD test was used to separate means ($P \leq 0.05$). The ANOVA was carried using the Statgraphics Centurion 18 software (ver. 18.1.13, Statgraphics Technologies Inc., Virginia, USA). Principal Component (PC) and Hierarchical Cluster (HC) analyses were performed on fatty acid profile using R Statistical Software (RStudio v1.4.1106, Boston, MA). As unsupervised methods, the groups of samples, obtained with both HC and PC analyses, can be observed even when there are no reference samples that can be used as a training set to establish the model. Principal component analysis (PCA) was carried out on the correlation matrix with the goal to reduce the dimensionality of the multivariate data of the matrix (14 samples x 8 variables), whilst preserving most of the variance. Means of all locations and years for each variable (= single fatty acid) have been used. The number of principal components (PCs) to retain is identified according to different criteria: Kaiser-Guttmann criterion (eigenvalues > 1), percentage of variance explained cumulatively; scree and elbow plot. Variable weights/loadings are examined to identify the variables that most contribute to each selected PC. Within each extracted component, the variables with the highest loadings (or weights) in absolute value are selected. The hierarchical

191 cluster analysis (HCA) was conducted on the normalized average values, with Ward's algorithm, using Euclidean distances
192 as a measure of (dis)similarity among the samples. Before applying hierarchical clustering method, to assess whether the
193 data are clusterizable, the Hopkins statistics (H) was used. A 0.64 H value was obtained indicating a good propensity of
194 our data to clusterize. If H < 0.5, the dataset is unlikely to have statistically significant clusters (Lawson et al., 1990;
195 Banerjee & Davé, 2004). The result was the dendrogram, a type of tree diagram showing hierarchical clustering
196 relationships between similar groups based on fatty acid content. In addition, the hierarchically clustered heatmap analysis
197 was performed on standardized data. It is also called a false coloured image, where data values were transformed to
198 colour scale.

199

200 **3. Results**

201 *3.1 Meteorological conditions and crop cycle length*

202 The safflower variety, CW99OL, confirmed its wide environmental plasticity and suitability to be grown as a winter crop in
203 north and central Italy, even in marginal land. It completed its growing cycle in about 190 day in Emilia Romagna, while
204 the cycle was significantly shorter in Tuscany (mean 145 day), but the differences in GDDs accumulated from sowing to
205 harvest were negligible across regions (~1850 GDD, Table 3). Thus, the variation in growing cycle duration, coupled with
206 similar GDD accumulation, was directly related to the differences in temperatures in the two regions with Tuscany being
207 much warmer, either in minimum and in maximum temperatures, than Emilia Romagna. The differences in temperatures
208 remained constants in the two growing seasons and were more pronounced in Tuscany than in Emilia Romagna. The trial
209 with the lowest mean minimum temperature was at Cadriano (ER) in GS2 (7.8°C), and the one with the highest minimum
210 temperature was at San Piero a Grado (TU) in GS1 (15.5°C). Concerning mean maximum temperature, the highest value
211 (25.6°C) was recorded at Larciano in GS2, and the lowest in Ozzano dell'Emilia 1-2-3 (19.3°C) in GS1. Precipitation varied
212 across regions and growing seasons: Emilia Romagna was drier than Tuscany, and the first growing season had less
213 precipitation than the second in both environments. Differences in the precipitation patterns within regions and growing
214 seasons were relevant particularly in the second growing season in Tuscany, and in both seasons in Emilia Romagna.
215 The driest trial was the one established at Cadriano in the second growing season, receiving about 140 mm from sowing
216 to harvest, while the wettest one was Santa Luce in the second growing season with more than 300 mm (Table 3). In

general, as expected, safflower cycle was shorter when the cumulative precipitation from sowing to harvest was lower and/or the sowing was delayed.

3.2 Morphological traits and agronomic performance of safflower

The ANOVA results are reported in Table 4. All the surveyed morphological and agronomic parameters, as well as the main FAs and FA groups, were significantly influenced by either the main factors (region R and/or growing season GS) and/or the interaction "R x GS", except for the total number of capitula per plant (HD), which did not significantly vary. Plant density (PD) at harvest was significantly ($P \leq 0.05$) influenced by the growing region and the interaction "R x GS" (Fig. 1). Concerning the growing region, safflower in Tuscany had almost the double plant density at harvest than in ER (45 vs. 29 plants m^{-2}, $P \leq 0.05$), this is probably linked to the different seeding rate adopted by the farmers, in relation to their practical knowledge. As regard the interaction "R x GS", safflower plant density in ER was similar between growing season, while in TU significant differences emerged between seasons with the second having 50 plants m^{-2} while in the first season the density was about 20% lower (Fig. 1). Plant height resulted significantly affected only by growing season (Table 4), and safflower was taller in the first season than in the second (1.04 vs. 0.87 m, $P \leq 0.05$). Concerning the branching pattern, it was significantly affected by the growing region and the interaction "R x GS" (Table 4). Safflower had more branches when grown in ER than in TU (5.4 vs. 4.5 branches $plant^{-1}$ as mean value over the two GS). Interestingly the branching pattern was not directly related to the plant density, since the interaction "R x GS" showed that the highest number of branches was surveyed in ER in the second growing season, and the lowest in ER in the first year and in TU in the second one, while TU in the first GS had an intermediate behavior (Fig. 1). The number of capitula per plant was on average 8.6 and resulted not affected by any of the factors considered (Table 4). Analyzing the agronomic performance of safflower in relation to straw and seed yield, the first one was significantly affected by growing region, while the second by "R x GS" interaction (Table 4). Concerning straw yield (Fig. 2A), safflower confirmed to be able to produce relevant aboveground biomass, producing on average 6495 kg DM ha^{-1} (grand mean), and in TU the straw production was significantly higher than in ER (7616 vs. 5142 kg DM ha^{-1}, $P \leq 0.05$). Safflower seed yield was on average 1775 kg DM ha^{-1}, and it resulted significantly higher in ER in GS1 than in all the other cases (Fig. 2B), producing on average about 50% higher seed yield. 1000-seed weight (TSW) was the only surveyed parameter significantly influenced by the two considered factors (R and GS) and their interaction (Table 4). Safflower seeds were heavier in ER than in TU (41.8 vs. 37.4 g, $P \leq$

244 0.05), and in the first than in the second season (40.8 vs. 38.2 g, $P \leq 0.05$). Concerning the "R x GS" interaction (Fig. 3),
245 seeds produced in TU in GS1 were the lightest, while the ones from ER in GS1 were the heaviest. Safflower seeds from
246 TU_GS2 and ER_GS2 presented intermediate values and did not differ in their weight in response to the growing
247 environment (Fig. 3). Seed oil content was significantly influenced by the growing region and the "R x GS" interaction
248 (Table 4). In TU safflower seeds were about 10% richer in oil compared with ER seeds (40.2 vs. 36.3% DM, TU vs. ER,
249 respectively, $P \leq 0.05$). When analyzing the "R x GS" interaction, four different means were evident with the highest seed
250 oil content in TU_GS2, followed by TU_GS1 and ER_GS1. The lowest oil content was observed in ER_GS2 (Fig. 4A). Oil
251 yield can be considered as one of the main attributes to compare the productive performance of safflower across growing
252 environments. In the present study, on average oil yield of 682 kg DM ha^{-1} (grand mean) was determined, with a coefficient
253 of variation of 0.48, and a significant "R x GS" interaction was surveyed (Table 4). Despite the higher oil content in the
254 seeds produced in Tuscany, the oil yield followed the same trend observed for seed yield, just with one small exception
255 (Fig. 4B). In details, the highest oil yield was observed in ER_GS1 and TU_GS2, but the latter not being different from the
256 oil produced by TU_GS1. The oil production in ER_GS2 was the lowest mean, but not different to TU_GS1 (Fig. 4B).

257

258 *3.3 Safflower oil quality*

259 With the scope of understanding which of the studied factors (i.e., region and/or growing season) significantly
260 affected safflower oil quality, the ANOVA was carried out for the main FAs characterizing safflower oil, i.e. oleic and linoleic
261 acid, and for the 3 FA groups, i.e. saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA).
262 Interestingly for all the considered oil quality traits, the growing region showed a significant effect, while the growing season
263 was never significant, and the "R x GS" interaction was significant only for linoleic acid, SFA, and PUFA (Table 4). Oleic
264 acid (C18:1) represented on average about 78% DM of safflower CW99OL fat, and oil from ER reached significantly higher
265 contents of C18:1 than the ones from TU (78.8 vs. 75.9% DM, in ER vs. TU respectively, $P \leq 0.05$, Table 5). Concerning
266 linoleic acid (C18:2), the mean content was 12.6% DM, and as expected there was an opposite behavior compared with
267 oleic since the samples from TU were richer in C18:2 than the ones from ER (15.7 vs. 11.6% DM, in TU vs. ER respectively,
268 $P \leq 0.05$, Table 5). The "R x GS" interaction reported in table 5 showed that in ER the first GS had the lowest C18:2
269 compared with the second, while in TU it was the opposite with the first having the higher value (Table 5). Concerning the
270 FA groups, safflower oil from ER had the highest amount of SFA and MUFA, while PUFA were promoted in samples from

Tuscany (Table 5). In particular, SFA and MUFA were 6% and 5% higher, respectively ($P \leq 0.05$) in ER than in TU, while PUFA showed great variability across the growing regions, with a remarkable +38% when safflower was grown in TU than in ER (Table 5). Interestingly for SFA and PUFA a significant interaction "R x GS" was surveyed (Table 4). For SFA the content in TU was stable across growing seasons, while in ER seeds from GS1 had a significant higher amount than those of GS2 ($P \leq 0.05$, Table 5). For PUFA, the seeds from TU_GS1 had the highest amount, but not different to the one from the same region in GS2, while in ER the trend was the opposite with the seeds of the first GS having the lowest amount, compared with the second one (Table 5).

In order to further investigate the environmental effects impacting on safflower oil quality, a PCA was carried out to possibly highlight factors grouping correlating qualitative characteristics (FA composition) of safflower seeds together, and then to identify clusters across environments and growing seasons. The first two principal components (PCs) explained a cumulative variance of 83.8%, on the base of the eigenvalue's comparison, with the eigenvalue weight of 4.67 and 2.04, for PC1 and PC2 respectively. PC1, which explained 58.3% of the total variance, was positively correlated with stearic (C18:0), oleic (C18:1), arachidic (C20:0), and lignoceric acid (C24:0), and negatively correlated with linoleic acid (C18:2). Principal component 2 (PC2), which explained 25.5% of the total variance, was positively correlated with eicosenoic acid (C20:1) and behenic acid (C22:0), while negatively correlated with palmitic acid (C16:0). The biplot of the PCA, reported in Figure 5, allowed to identify three main groupings based on their similarity in terms of FA content: the first represented by sample 4 (= CA_GS1); the second group by samples from TU locations, i.e. samples 8 (= FA_GS1), 9 (= SPG_GS1), 10 (= SL_GS1), 12 (= FA_GS2), 13 (= SPG_GS2) and 14 (= SL_GS2). Finally, the third grouping represented by the samples from the ER locations, i.e. samples 1 (= OZ2_GS1), 2 (= OZ3_GS1), 3 (= OZ1_GS1), 5 (= OZ2_GS2), 6 (= OZ3_GS2) and 7 (= CA_GS2) to which sample 11 (= LA_GS2) from TU is added. In detail, the first group was characterized by the highest content of palmitic acid (C16:0) and the lowest content of behenic acid (C22:0). The second group included the samples characterized mainly by the highest level of linoleic acid with three sub-groups (samples 8 and 12 for the first group; samples 9 and 14 for the second, and samples 10 and 13 for the third group) in relation to their greater or intermediate content. Finally, the third group clustered the samples with the highest contents of C18:0, C18:1, C20:0 and C24:0 with the creation of two subgroups (samples 2 and 6; and samples 1, 3, 5, 7, and 11, respectively) in relation to their greater or intermediate content. In particular, the sample 11, coming from TU, is much more similar to the ER samples for the FA profile and especially for a lower content in linoleic acid. These observations have been confirmed by HCA. The two-way dendrogram of the HCA is reported in Figure 6. Through the heatmap representation, it is possible to

299 simultaneously visualize clusters of samples (locations) and variables (= FAs) and to find the variables that appeared to
300 be characteristic for each sample cluster. Among the FAs, C16:0, C24:0, C18:1, C20:0 and C18:0 were clustered together,
301 while C20:1, C22:0, and C18:2 were grouped in the second macro-cluster. Regarding samples (Fig. 6), the first macro-
302 cluster (red) was grouped by itself, while the second comprised two sub-clusters (green and blue). Based on their FA
303 content, sample in the red cluster shared the highest C16:0 content and the lowest C22:0 content; samples of the green
304 macro-cluster, all from TU locations, were, instead, characterized mainly by highest content of C18:2, and, finally, samples
305 of the blue cluster, all from ER except for sample 11 (from TU) exhibited the greatest content of C18:0, C18:1, C20:0 and
306 C24:0 and the lowest one of linoleic acid. As previously described, the dendrogram also shows more clearly, the formation
307 of subgroups given by the high or intermediate content of each FA.
308

309 **4. Discussion**

310 Mediterranean farmers, mostly relying on winter cereals, generally in monoculture, are searching for low input and
311 alternative winter crops that can diversify their current cropping systems and promote agricultural sustainability. Cropping
312 system diversification is one of the main principles of agroecological transition, although local references about potential
313 new species, such as alternative oilseed crops, is almost lacking, particularly under real on-farm conditions. So, in the
314 present study, high-oleic safflower was evaluated, for the first time, at farm level, across different environments and
315 growing seasons in north and central Italy with the aim to demonstrate its feasibility as promising winter crop at farm level.
316 Overall, the obtained results showed as CW99OL safflower adequately adapted to the pedo-climatic conditions of the
317 study areas, with good agronomic performances in terms of seed and biomass yield, as well as oil content and quality. In
318 the test environments, winter safflower completed its growth cycle in 190 and 145 d (in ER and TU, respectively), in line
319 with that observed by La Bella et al. (2019) in southern Italy testing different winter-sown genotypes. Despite the
320 differences in the number of days to complete the crop cycle between the two regions, the GDD accumulated to reach
321 maturity seemed not to be influenced by the environment/location (~1850 GDD), which make thermal time a useful
322 predictor of safflower maturity and harvest time. In both environments, the safflower yield range was consistent with
323 previous values reported in the literature (Koutroubas and Papakosta, 2010; La Bella et al., 2019; Abou Chehade et al.,
324 2022), with an average value of 1775 kg DM ha^{-1}. However, significant differences in terms of morphological and yield
325 parameters were surveyed between the two regions in the two growing seasons to confirm as environmental conditions,

326 together with cultivation practices at farm level, can significantly influence the yield performances of the crop. The present
327 observations, in fact, underlined as weather conditions significantly affected seed yields of safflower, despite its high
328 rusticity and adaptability to various environments. The safflower outperformance in ER in the first season could be ascribed
329 to the earlier sowing (~ 50 days) which lead to a prolonged vegetative development, which might have affected the
330 remobilization of photosynthates during seed filling stage, thus significantly increasing 1000-seed weight (Fig. 3). In
331 addition, the earlier sowing performed in ER in comparison with TU conducted safflower to develop under milder
332 temperatures (Table 3), which may have alleviated the occurrence of abiotic stress during the critical reproductive phase.
333 In fact, despite safflower is considered to be tolerant to drought and heat, flowering remains the most sensitive stage to
334 environmental stresses (Abou Chehade et al., 2022). Several studies reported how hot and dry conditions during the
335 growing season may negatively influence the seed yield of safflower (Mohammadi et al., 2018; Koutroubas et al., 2021).
336 Concerning biometric characteristics and yield components, the present findings showed as branching pattern was
337 significantly affected by the growing region and "R x GS" interaction, confirming that this trait is, not only genetically, but
338 also environmentally regulated (Weiss, 2000). In addition, previous studies (Koutroubas et al., 2004; Santos et al., 2017)
339 demonstrated that the dry matter accumulation is strongly correlated with plant height and branching degree, even if the
340 present results did not seem to confirm this relation. Also, straw yield was significantly affected by growing region, with the
341 highest value achieved in TU (7616 vs. 5142 kg ha^{-1}, TU vs ER, respectively). As reported by Abou Chehade et al. (2022),
342 straw removed high amounts of macronutrients such as N and P, that can return into the soil once they are incorporated
343 with tillage, promoting SOM storage for the following crops although via slow mineralization (C/N ranging from 46 to 85,
344 according to La Bella et al., 2019).

345 Seed oil content and oil yield represent the main traits for comparing the productive performance of safflower
346 across growing environments, since they are useful for evaluating the real possibility of introducing this crop into a new
347 environment/cropping system. Generally, as for many other oilseed crops, safflower oil content is strongly affected by
348 genetic characteristics and pedo-climatic conditions of the cultivation site, as well as by the applied agronomic
349 management. In the present on-farm trials, oil content was significantly affected by growing region and by "R x GS"
350 interaction. Considering the effect of the growing region, in both the cumulative precipitation was higher in GS1 than in
351 GS2 (Table 3), but the milder temperatures occurred in ER during GS1, particularly in OZ1-2-3, promoted seed oil content,
352 and the same response behavior was surveyed in TU in GS2, which was characterized by lower temperature, particularly
353 minimum ones. Sehgal et al. (2018) underlined that, in safflower, a decline in oil accumulation occurred under low water

354 availability and high air temperatures, due to their negative effects on the enzymes involved in the conversion of
355 carbohydrates to lipids. Depending on the growing region, in the present trials oil content ranged between 36.6% and
356 40.2%, close to those previously reported in Mediterranean areas (La Bella et al., 2019; Koutroubas et al., 2021; Abou-
357 Chehade et al., 2022). Oil yield, determined by the product of seed oil content and seed yield, followed the same trend
358 observed for the seed yield with the highest value for ER_GS1 (904 kg ha^{-1}). The high quality of safflower oil, in terms of
359 fatty acid composition, biological activities, hedonic properties and high stability at elevated temperature, has made it an
360 attractive feedstock for multiple biobased applications (Asgarpanah and Kazemivash 2013; Nazir et al., 2021). In particular,
361 safflower oil with high oleic acid content (>75%) has a greater economic value for both food and non-food uses, thanks to
362 its higher oxidative stability compared with typical safflower oil, characterized by a higher rate of polyunsaturated fatty
363 acids (Nazir et al., 2021; Nogales-Delgado et al., 2021). Safflower fatty acid composition is under genetic control but also
364 highly affected by the prevailing meteorological conditions occurring during crop cycle, particularly during seed filling stage
365 (Roche et al., 2019; Zemour et al., 2021; Abou-Chehade et al, 2022;). Although the "high oleic" trait has been reported as
366 environmentally stable and genetically controlled (Hamdan et al., 2009), the present results highlighted significant
367 differences on C18:1 content in response to growing region. In details, the present findings showed that safflower oil from
368 TU exhibited higher C18:2 and PUFA content compared with ER one. The latter, conversely, exhibited the highest C18:1,
369 MUFA and SFA content. As suggested by the biplot PCA, the differences in fatty acid composition were less due to the
370 growing season (GS) and more to the growing region (TU vs. ER). This was further confirmed by the 2-way dendrogram
371 that showed the formation of 3 macro-clusters (1 = grouped only CA_GS1 from ER; 2 = grouped all TU locations; 3 =
372 grouped the remaining ER locations) related to FA profile. Among these FAs, mainly C18:1, C18:2, and SFAs appeared
373 to be characteristic for each macro-cluster. The effect of environmental conditions on C18:1 content in high-oleic safflower
374 varieties has been poorly investigated in the literature. In sunflower, high oleic hybrids showed differences in their response
375 to the environment in terms of oleic acid accumulation (Triboï-Blondel et al., 2000; Luquez et al.,2002; Roche et al., 2006).
376 On the contrary, several studies, carried out on "traditional" safflower varieties have reported different responses of single
377 fatty acids to specific environmental conditions, such as high temperature and water availability during seed maturation.
378 Although the effect of the environment on fatty acid composition in high-oleic safflower varieties seemed more restrained
379 than in traditional ones, it can still be a concern, especially when the oil has to fulfill strict quality standards to meet specific
380 industrial end-uses. Definitively, this on-farm study demonstrated as high oleic safflower can be an interesting opportunity
381 for Mediterranean farmers who are willing to differentiate their rotations while producing an oilseed crop with several

biobased applications. Even in marginal conditions (i.e., FA, SL, OZ-2 and OZ3 sites), winter safflower confirmed to be a versatile oilseed crop, able to provide satisfactory seed yield, and interesting advantages compared with sunflower, such as an early soil cover with reduced risk of N-leaching and soil erosion.

Conclusion

Safflower, and in particular when grown with a winter cycle, appeared to be a feasible alternative to winter cereal monoculture for northern and central Italy. Particularly when considering that the present study includes only on-farm trials, carried out by local farmers, who were for the first time approaching this new oilseed crop. Thus, the value of the present study, beside the promising productive results achieved, is for demonstrating how easy could be the technical scale up of a crop like safflower. This might represent an important and unique trait of this new oilseed crop, compared with others suitable for Italy. Nevertheless, some attention should be paid in understanding the real attitude of specific environments in sourcing oil with fatty acid composition more in line with the request of the biobased industry. Furthermore, it's worth mentioning that a thorough study on the effects of safflower inclusion in typical crop rotations of tested regions is urgently needed for the industrial scale-up of this promising crop. Despite being safflower seed yield not affected by growing environment, 1000-seed weight, seed oil content, and oleic acid content were promoted in Emilia Romagna, resulting this region more suitable for its cultivation. On the other hand, when in Tuscany earlier sowing was possible (GS2) safflower performance was similar than in ER, thus confirming how also this area can be highly suitable for winter safflower cultivation applying an optimized agronomic management.

CRediT authorship contribution statement

Federica Zanetti: Formal analysis, Writing – original draft, Writing – review & editing; Luciana G. Angelini: Conceptualization, Project administration, Supervision, Validation, Resources, Writing – original draft, Writing – review & editing; Sara Berzuini: Investigation; Methodology, Data curation, Writing – original draft, Writing – review & editing; Lara Foschi: Methodology, Data curation, Writing – review & editing; Clarissa Clemente: Formal analysis, Methodology, Writing – review & editing; Federico Ferioli: Formal analysis, Methodology, Writing – review & editing; Angela Vecchi: Investigation, Data curation; Alessandro Rossi: Investigation, Data curation; Andrea Monti: Project administration, Supervision,

Methodology, Validation, Resources, Writing – review & editing; Silvia Tavarini: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Description of the soil tillage, sowing method, fertilization and sowing and harvest dates at each location and growing season (GS).

Location	*Soil Tillage*	*Sowing method*	*Fertilization*	*Sowing date*		*Harvest time*	
				GS1	*GS2*	*GS1*	*GS2*
FA	Rotary harrow	Cereal seeder dist. 0.15 m	Organic fertilizer (pre-sowing/21 kg N ha^{-1}; 39 kg P_2O_5 ha^{-1})	28 Mar	14 Mar	29 Jul	5 Aug
LA	Plowing (0.3 m depth) + vibro-cultivator and rotary harrow x2	Cereal seeder dist. 0.12 m	Organic fertilizer (pre-sowing/30 kg N ha^{-1})	-	17 Mar	-	3 Aug
SL	Plowing (0.3 m depth) + 2 disk harrows + disk arrow	Cereal seeder dist. 0.14 m	None	23 Feb	24 Jan	30 Jul	27 Jul
SPG	Plowing (0.3 m depth) + disk harrow + rotary harrow	Plot seeder dist. 0.15 m	Ammonium nitrate (top dressing/30 kg N ha^{-1}	4 Feb	19 Mar	21 Aug	4 Aug
CA	Plowing (0.3 m depth) + disk harrow + rotary harrow	Cereal seeder dist. 0.45 m	Organic fertilizer (pre-sowing 25 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, top dressing 30 kg N ha^{-1})	16 Jan	20 Jan	1 Aug	22 Jul
OZ-1	Rotary harrow (0.3 m depth)	Cereal seeder dist. 0.45 m	Organic fertilizer (pre-sowing 25 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, top dressing 30 kg N ha^{-1})	7 Jan	-	17 Jul	-
OZ-2	Rotary harrow (0.3 m depth)	Cereal seeder dist. 0.45 m	Organic fertilizer (pre-sowing 25 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, top dressing 30 kg N ha^{-1})	7 Jan	21 Jan	17 Jul	02 Aug
OZ-3	Rotary harrow (0.3 m depth)	Cereal seeder dist. 0.45 m	Organic fertilizer (pre-sowing 25 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, top dressing 30 kg N ha^{-1})	7 Jan	21 Jan	17 Jul	02 Aug

520 Table 2. Locations, coordinates and main soil characteristics of the study sites in the two growing seasons.

Region		Tuscany				Emilia-Romagna			
Location		Santa Luce	Fauglia	San Piero a Grado	Larciano	Ozzano plane	Ozzano 15% slope	Ozzano 25% slope	Cadriano
Site ID		SL	FA	SPG	LA	OZ-1	OZ-2	OZ-3	CA
Coordinates		43°27'N, 10°31'E	43°34'N, 10°30'E	43°40'N, 10°18'E	43°47'N, 10°49'E	44°26'N 11°28'E	44°24'N 11°28'E	44°24'N 11°28'E	44°33'N, 11°23'E
Altitude	(m a.s.l)	63	59	19	0	67	115	230	33
Slope	(%)	15	20	0	0	0	15-20	25-30	0
Texture[1]		CL	SL	L	SL	CL	CL	L	L
pH		8.35	6.60	8.08	6.15	7.92	8.08	8.08	8.07
Organic matter	(%)	1.66	2.20	1.63	1.36	1.99	1.33	0.99	1.82
Total Nitrogen	(‰)	1.60	1.35	0.84	0.99	1.44	0.87	1.06	1.31
Avail. Phosphorus	(ppm)	8.29[OI]	1.49[B]	13.42[OI]	3.07[B]	22 [OI]	16 [OI]	22[OI]	21[OI]
Exch. Potassium	(ppm)	211	215	106	108	194	213	198	181

521 [1]CL, SL, L refer to clay loam, sandy loam, and loam, respectively.

522 [OI] Olsen method.

523 [B]Bray method.

524

Table 3. Mean minimum and maximum temperatures, cumulative precipitation, growing degree days (GDD) and days from sowing to harvest across the different test locations in Tuscany and Emilia Romagna in the two growing seasons (GS).

		GS1[a]					*GS2*[a]				
Region	*Site ID*	Mean Tmin (°C)	Mean Tmax (°C)	Prec (mm)	GDD[b]	Days	Mean Tmin (°C)	Mean Tmax (°C)	Prec (mm)	GDD[b]	Days
Tuscany	*FA*	13.7	24.8	295.4	1752	123	12.0	24.2	207.8	1900	143
	LA	-	-	-	-	-	11.3	25.6	201.6	1874	138
	SL	11.2	22.5	244.2	1861	157	10.0	21.1	307.0	1967	184
	SPG	15.5	23.7	290.6	2061	141	14.1	22.2	200.4	1818	137
	Tuscany mean	13.4	23.6	276.7	1891	140	11.8	23.3	229.2	1889	150
Emilia-Romagna	*CA*	9.2	22.2	251.6	1988	198	7.8	20.8	143.4	1776	183
	OZ 1-2-3	8.3	19.3	156.4	1675	192	9.6	20.8	212.8	1956	193
	Emilia Romagna mean	8.7	20.7	204.0	1831	194	8.7	20.8	178.1	1866	188

[a]GS= in Tuscany GS1 and GS2 corresponded to 2019 and 2020, respectively, while in Emilia Romagna GS1 and GS2 corresponded to 2020 and 2021, respectively.

[b]Base temperature for GDD calculation 5°C (Mirshekari et al., 2013)

Table 4. ANOVA table with *F*-values and statistical significance for the agronomic, morphological and seed qualitative traits surveyed in the multi-year multi-location trial with high-oleic safflower in Italy. Considered factors: region (R) and growing season (GS). Each region, i.e. Emilia Romagna vs. Tuscany, includes 4 test locations, namely for Emilia Romagna: OZ- 1,2,3 and CA, for Tuscany: FA, LA, SL, and SPG (see tables 1-3). Considered parameters: PD - final plant density (pp m^{-2}); PH - final plant height (m); straw - straw yield (kg DM ha^{-1}); SY - seed yield (kg DM ha^{-1}); BP - number of main branches per plant; CD - total number of capitula per plant; TSW - 1000-seed weight (g); OIL - seed oil content (% DM); OY – oil yield (kg DM ha^{-1}); C18:1 - oleic acid content (% DM), C18:2 – linoleic acid content (%DM); SFA – saturated fatty acids (%DM); MUFA – monounsaturated fatty acids (%DM); PUFA – polyunsaturated fatty acids (%DM).

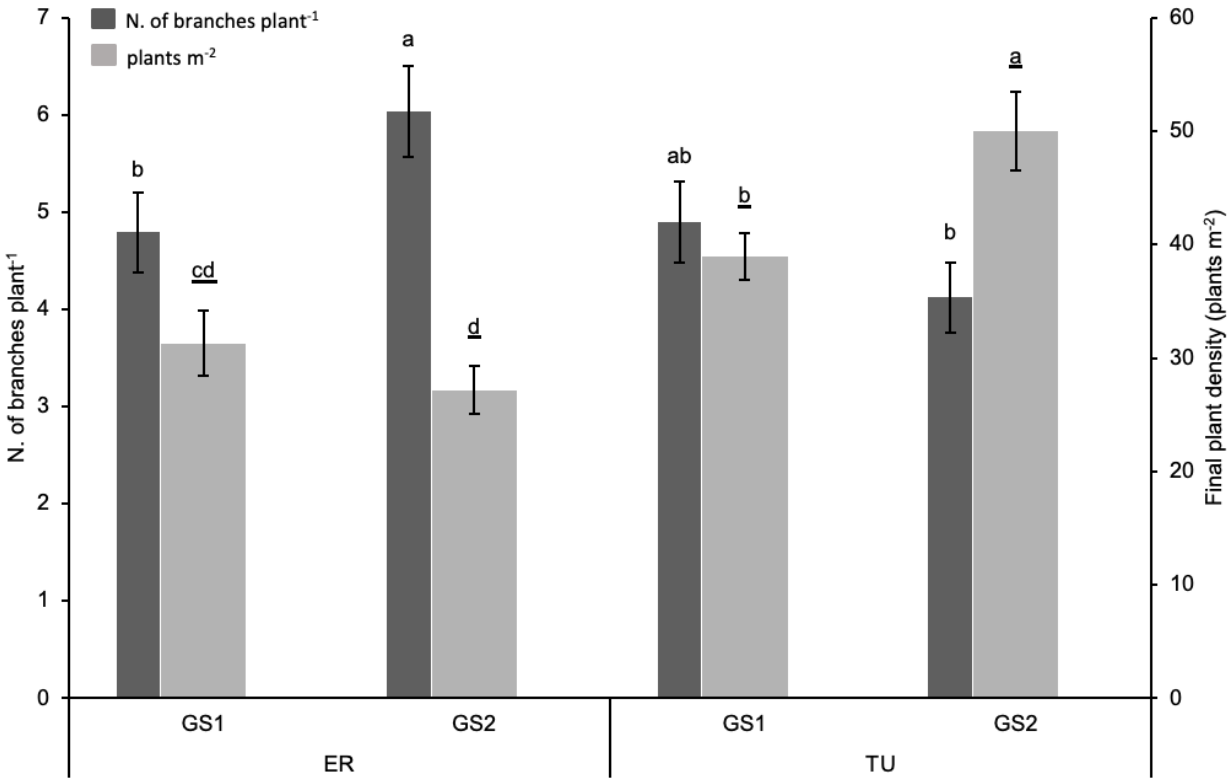
Source of variation	PD	PH	BP	CD	Straw	SY	TSW	OIL	OY	C18:1	C18:2	SFA	MUFA	PUFA
R	26.1**	3.75^{ns}	4.51*	1.19^{ns}	11.3**	3.77^{ns}	15.8**	101.8**	3.61^{ns}	136.3**	228.3**	36.5**	267.4**	261.3**
GS	1.36^{ns}	11.3**	0.29^{ns}	0.28^{ns}	2.31^{ns}	1.06^{ns}	51.0**	0.36^{ns}	0.06^{ns}	0.11^{ns}	0.40^{ns}	2.54^{ns}	0.53^{ns}	0.43^{ns}
R x GS	6.48*	0.13^{ns}	5.67*	1.19^{ns}	0.00^{ns}	12.1**	45.4**	13.4**	14.69**	3.47^{ns}	5.71*	5.16*	1.96^{ns}	5.61*

*, ** Significant at the 0.05, 0.01 probability levels, respectively (LSD Fishers' test); ns = not significant.

Table 5. Results (mean values ± standard error) on the main fatty acids characterizing safflower oil, i.e. oleic (C18:1) and linoleic acid (C18:2), and fatty acid groups, i.e., SFA (Saturated fatty acids), MUFA (Monounsaturated fatty acids), PUFA (Polyunsaturated fatty acids) in response to the main effect growing region (ER = Emilia Romagna, TU = Tuscany), and the interaction "region x growing season" in the multi-year multi location study carried out in Italy. Different letters: statistically different means within the same fatty acid or fatty acid group for the main effect growing region for $P \leq 0.05$ (LSD Fisher test). Different italic letters: statistically different means within the same fatty acid or fatty acid group for the interaction effect "region x growing season" for $P \leq 0.05$ (LSD Fisher test). Ns= not significant

Main effect		*C18:1*	*C18:2*	*SFA*	*MUFA*	*PUFA*
Region						
ER		78.8^{a}±0.07	11.6^{b}±0.08	8.3^{a}±0.04	80.1^{a}±0.07	11.6^{b}±0.08
TU		75.9^{b}±0.33	15.7^{a}±0.37	7.8^{b}±0.08	76.3^{b}±0.33	16.0^{a}±0.37
Interaction "GS x Region"						
Region	*Growing season*[1]	*C18:1*	*C18:2*	*SFA*	*MUFA*	*PUFA*
ER	GS1	ns	11.4^{b}±0.10	8.4^{a}±0.04	ns	11.4^{b}±0.10
	GS2	ns	11.8^{b}±0.13	8.1^{b}±0.05	ns	11.8^{b}±0.13
TU	GS1	ns	16.1^{a}±0.31	7.8^{c}±0.13	ns	16.4^{a}±0.31
	GS2	ns	15.3^{a}±0.71	7.8^{c}±0.10	ns	15.6^{a}±0.70

[1] GS= in Tuscany GS1 and GS2 corresponded to 2019 and 2020, respectively, while in Emilia Romagna GS1 and GS2 corresponded to 2020 and 2021, respectively.



553

554 Figure 1. The number of branches per plant, on the left axis, and final plant density (plants m^{-2}), on the right axis, surveyed
555 in the multi-year and multi-location trial on safflower in response to the interaction between region (ER = Emilia Romagna
556 vs. TU =Tuscany) and growing season (GS1 vs. GS2). Vertical bars: standard error. Different letters: significant different
557 means $P \leq 0.05$ (LSD Fisher test) for number of branches per plant. Different underlined letters: significant different means
558 $P \leq 0.05$ (LSD Fisher test) for final plant density.

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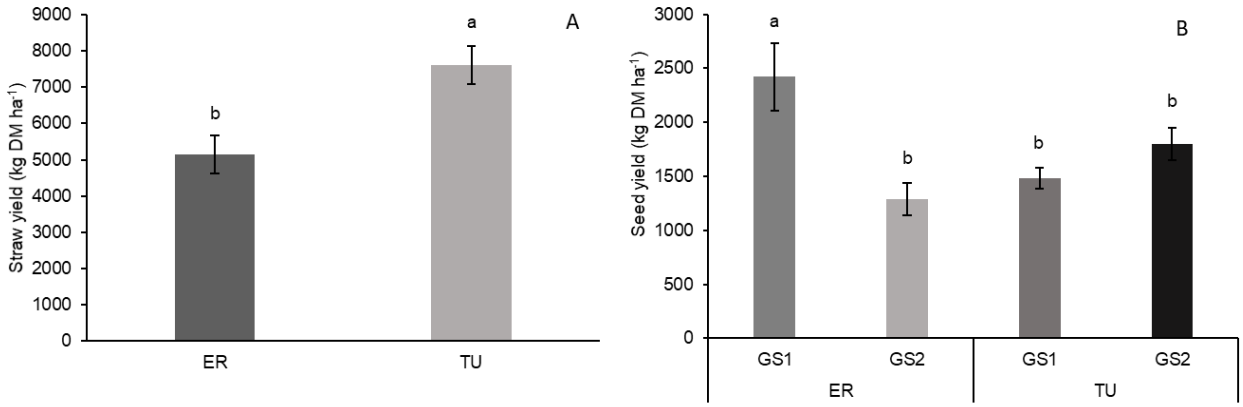


561

562 Figure 2. A) Safflower straw yield (kg DM ha^{-1}) in the multi-year and multi-environment in response to the main effect
563 region (ER = Emilia Romagna vs. TU = Tuscany). Vertical bars: standard error. Different letters: significant different means
564 $P \leq 0.05$ (LSD's Fisher test). B) Safflower seed yield (kg DM ha^{-1}) in the multi-year and multi-environment in response to
565 the interaction in response to the interaction between region (ER = Emilia Romagna vs. TU =Tuscany) and growing season
566 (GS1 vs. GS2). Vertical bars: standard error. Different letters: significant different means $P \leq 0.05$ (LSD Fisher test).

567

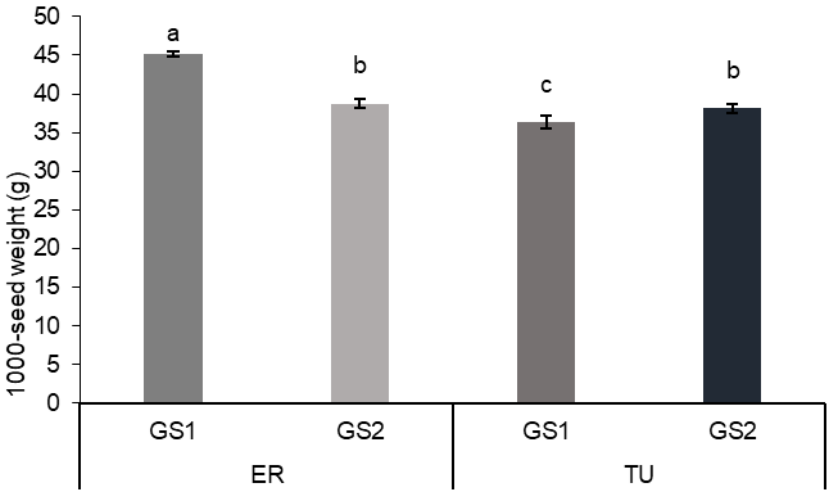
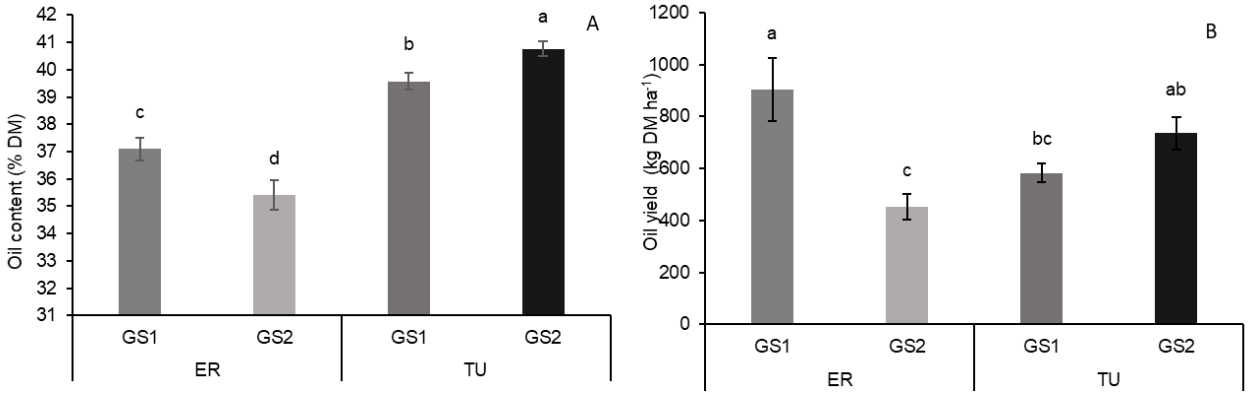


Figure 3. Safflower 1000-seed weight (g) in the multi-year and multi-environment in response to the interaction in response to the interaction between region (ER = Emilia Romagna vs. TU =Tuscany) and growing season (GS1 vs. GS2). Vertical bars: standard error. Different letters: significant different means $P \leq 0.05$ (LSD Fisher test).



574

575 Figure 4. A) Safflower seed oil content (%DM) in the multi-year and multi-environment in response to the interaction in

576 response to the interaction between region (ER = Emilia Romagna vs. TU =Tuscany) and growing season (GS1 vs. GS2).

577 Vertical bars: standard error. Different letters: significant different means $P \leq 0.05$ (LSD Fisher test). B) Safflower oil yield

578 (kg DM ha^{-1}) in the multi-year and multi-environment in response to the interaction in response to the interaction between

579 region (ER = Emilia Romagna vs. TU =Tuscany) and growing season (GS1 vs. GS2). Vertical bars: standard error.

580 Different letters: significant different means $P \leq 0.05$ (LSD Fisher test).

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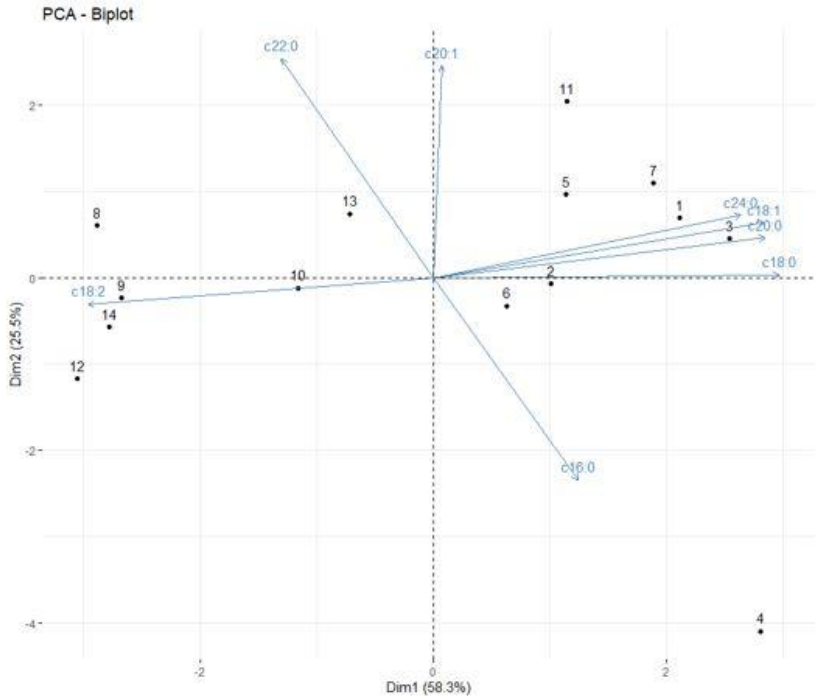
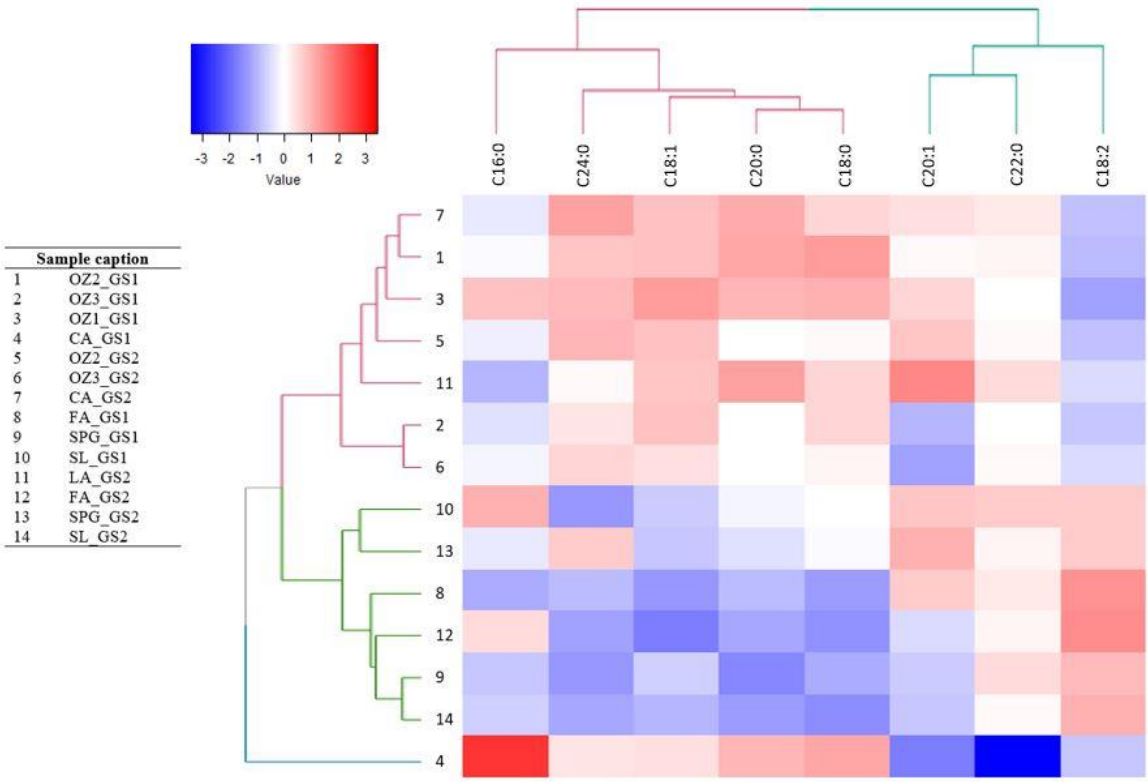


Sample caption	
1	OZ2_GS1
2	OZ3_GS1
3	OZ1_GS1
4	CA_GS1
5	OZ2_GS2
6	OZ3_GS2
7	CA_GS2
8	FA_GS1
9	SPG_GS1
10	SL_GS1
11	LA_GS2
12	FA_GS2
13	SPG_GS2
14	SL_GS2

586

587 Figure 5. PCA Biplot (score plot + loading plot) for PC1 and 2, describing variation in fatty acid composition of safflower
588 seeds grown in different environments and growing seasons. Each sample represents a location and a growing season
589 as explained in sample caption.

590



Sample caption	
1	OZ2_GS1
2	OZ3_GS1
3	OZ1_GS1
4	CA_GS1
5	OZ2_GS2
6	OZ3_GS2
7	CA_GS2
8	FA_GS1
9	SPG_GS1
10	SL_GS1
11	LA_GS2
12	FA_GS2
13	SPG_GS2
14	SL_GS2

591

592 Figure 6. Hierarchically Clustered Heatmap on fatty acid composition of safflower seeds grown in different environments
593 and growing seasons. Each sample represents a location and a growing season as explained in sample caption. Data
594 values were transformed to color scale.

595

CRediT authorship contribution statement

Federica Zanetti: Formal analysis, Writing – original draft, Writing – review and editing; **Luciana G. Angelini**: Conceptualization, Project administration, Supervision, Validation, Resources, Writing – original draft, Writing – review and editing; **Sara Berzuini**: Investigation; Methodology, Data curation, Writing – original draft, Writing – review and editing; **Lara Foschi**: Methodology, Data curation, Writing – review and editing; **Clarissa Clemente**: Formal analysis, Methodology, Writing – review and editing; **Federico Ferioli**: Formal analysis, Methodology, Writing – review and editing; **Angela Vecchi**: Investigation, Data curation; Alessandro Rossi: Investigation, Data curation; **Andrea Monti**: Project administration, Supervision, Methodology, Validation, Resources, Writing – review and editing; **Silvia Tavarini**: Conceptualization, Writing – original draft, Writing – review and editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: